Amendments to the Claims

This listing of claims will replace all prior versions, and listings, of claims in the application:

Listing of Claims:

1. (Previously Presented) A method for detecting the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising:

(a) treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

(i) methylated form; or

(ii) non-methylated form

to pair with a nucleotide normally not pairing with said nucleotide prior to conversion;

(b) amplifying said nucleic acid molecule treated with said agent via at least one amplification primer to produce an amplification product and converting said amplification product into single stranded amplified nucleic acid molecules, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of said single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid molecule;

(c) real-time sequencing said single stranded amplified nucleic acid molecule; and

(d) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample.

2. (Original) The method of claim 1 wherein said sample is derived from a tissue, a body fluid or stool.

3. (Original) The method of claim 2 wherein said tissue is a tumor tissue, neurodegenerative tissue or a tissue affected with another neurological disorder.

4. (Previously Presented) The method of claim 1 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

5. (Previously Presented) The method of claim 1 wherein in (b) the nucleic acid molecule is amplified via LCR or PCR.

6. (Cancelled)

7. (Previously Presented) The method of claim 1 wherein said amplification primer is labeled with (a) biotin, (b) avidin, (c) streptavidin or (d) a derivative of (a), (b) or (c) or a magnetic bead.

8. (Previously Presented) The method of claim 1 wherein said nucleotide of (a)(i) is an adenine, guanine or a cytosine.

9. (Previously Presented) The method of claim 1 wherein said real-time sequencing comprises:

(a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;

(b) addition of a DNA polymerase, a ATP sulfurylase, a luciferase, an apyrase, adenosine-phosophosulfate (APS) and luciferin;

(c) sequential addition of dATP, dCTP, dTTP and dGTP;

(d) detection of a luminescent signal wherein an intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide at said predetermined position.

10. (Previously Presented) The method of claim 1, further comprising calculating a frequency of methylated nucleotides from results of said real-time sequencing.

11. (Previously Presented) The method of claim 1 wherein said agent suitable for the conversion of said nucleotide to pair with nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite.

12. (Previously Presented) A method for the diagnosis of a pathological condition or the predisposition for a pathological condition comprising detection of the methylation status of a nucleotide at a predetermined position in a nucleic acid molecule comprising:

(a) treating a sample comprising said nucleic acid molecule in an aqueous solution with an agent suitable for the conversion of said nucleotide if present in

(i) methylated form; or

(ii) non-methylated form

to pair with a nucleotide normally not pairing with a said nucleotide prior to conversion;

(b) amplifying said nucleic acid molecule treated with said agent via at least one amplification primer to produce an amplification product and converting the amplification product into single stranded amplified nucleic acid molecules, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of said single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid molecule;

(c) real-time sequencing said single stranded amplified nucleic acid molecule; and

(d) detecting whether said nucleotide is methylated or not methylated at said predetermined position in the sample to diagnose said pathological condition or the predisposition for said pathological condition.

13. (Previously Presented) The method of claim 12 wherein said pathological condition is cancer, a neurodegenerative disease or another neurological disorder.

14. (Original) The method of claim 13 wherein said cancer is a primary tumor, a metastasis or a residual tumor.

15. (Original) The method of claim 14 wherein said primary tumor is a glioma.

16. (Previously Presented) The method of claim 15 wherein said glioma is an astrocytoma, oligodendroglioma, an oligoastrocytoma, a glioblastoma, or a pilocytic astrocytoma.

17. (Previously Presented) The method of claim 38 wherein said neurodegenerative disease is
Alzheimer's disease, Parkinson disease, Huntington disease, or Rett-Syndrome.

18. (Previously Presented) The method of claim 38 wherein said neurological disorder is
Prader-Willi-Syndrome, Angelman-Syndrome, Fragile-X-Syndrome, or ATR-X-Syndrome.

19. (Previously Presented) The method of claim 12 wherein said nucleic acid molecule is a DNA molecule or an RNA molecule.

20. (Previously Presented) The method of claim 12 wherein in (b) the nucleic acid molecule is amplified via LCR or PCR.

21. (Cancelled)

22. (Previously Presented) The method of claim 12 wherein said amplification primer is labeled with (a) biotin, (b) avidin, (c) streptavidin or (d) a derivative of (a), (b) or (c) or a magnetic bead.

23. (Previously Presented) The method of claim 12 wherein said nucleotide of (a)(i) is an adenine, guanine or a cytosine.

24. (Previously Presented) The method of claim 12 wherein said real-time sequencing comprises:

(a) hybridization of a sequencing primer to said amplified nucleic acid molecule in single-stranded form;

(b) addition of a DNA polymerase, a ATP sulfurylase a luciferase, an apyrase, adenosine-phosphosulfate (APS) and luciferin;

(c) sequential addition of dATP, dCTP, dTTP and dGTP;

(d) detection of a luminescent signal wherein the intensity of the luminescent signal is correlated with the incorporation of a specific nucleotide at a specific position in the nucleic acid molecule and wherein the intensity of said signal is indicative of the methylation status of said nucleotide at said predetermined position.

25. (Previously Presented) The method of claim 12 further comprising calculating a frequency of methylated nucleotides from results of said real-time sequencing.

26. (Previously Presented) The method of claim 12

wherein said agent suitable for the conversion of said nucleotide to pair with a nucleotide normally not pairing with said nucleotide is a bisulfite, preferably sodium bisulfite.

27. (Previously Presented) The method of claim 1 wherein said method is a high-throughput method.

28. (Previously Presented) The method of claim 12 wherein said sample is derived from tissue, a body fluid or stool.

29. (Previously Presented) The method of claim 28 wherein said body fluid is blood, serum or urine.

30. (Previously Presented) The method of claim 1 wherein said nucleotide is a cytosine and is part of one of the following sequences: CpG, CpNpG or CpNpN.

31. (Previously Presented) The method of claim 1, wherein the methylation status of more than one predetermined nucleotide is detected and a number of samples are analyzed at the same time.

32. (Previously Presented) A method for generating new nucleotide pairing partners upon amplification of at least one nucleic acid molecule for the detection of the methylation status of nucleotides of said nucleic acid molecule, said method comprising:

a. providing said at least one nucleic acid molecule;
b. treating said nucleic acid molecule with an agent suitable for conversion of a nucleotide if present in methylated form or non-methylated form to

pair with nucleotide pairing partners normally not pairing with said nucleotide prior to conversion;

c. amplifying said nucleic acid molecule via at least one amplification primer to produce an amplification product and converting the amplification product into a single stranded nucleic acid molecules, wherein said at least one amplification primer is detectably labeled with a detectable label that forms an anchor for removal of said single stranded amplified nucleic acid molecules to generate a single stranded amplified nucleic acid molecule comprising said new nucleotide pairing partners normally not pairing with said nucleotide prior to conversion and;

d. real-time sequencing said single stranded amplified nucleic acid molecule;

e. determining the amount of said nucleotide pairing with said new nucleotide pairing partners to detect the methylation status of nucleotides of said nucleic acid molecule.

33. (Previously Presented) The method of claim 1, wherein the methylation status of more than one predetermined nucleotide is determined.

34. (Previously Presented) The method of claim 10, further comprising detecting an allele frequency, wherein an allele frequency of 5% can be detected.

35. (Previously Presented) The method of claim 1, wherein said primer does not comprise CpG.

36. (Previously Presented) The method of claim 1, wherein all nucleotides formerly methylated or not methylated in said nucleic acid molecule are detected.

37. (Currently Amended) The method of claim 8, wherein said ~~methylated~~ nucleotide of (a)(i) is an adenine or guanine.

38. (Previously Presented) The method of claim 12, wherein said pathological condition is a neurodegenerative disease or another neurological disorder.

39. (Previously Presented) The method of claim 34, wherein the allele frequency of 5% is detected with a standard deviation of not more than 1%.